124. Good Laboratory Practice Regulations: Second Edition, Revised and Expanded, *edited by Sandy Weinberg*
125. Parenteral Quality Control: Sterility, Pyrogen, Particulate, and Package Integrity Testing: Third Edition, Revised and Expanded, *Michael J. Akers, Daniel S. Larrimore, and Dana Morton Guazzo*
126. Modified-Release Drug Delivery Technology, *edited by Michael J. Rathbone, Jonathan Hadgraft, and Michael S. Roberts*
127. Simulation for Designing Clinical Trials: A Pharmacokinetic-Pharmacodynamic Modeling Perspective, *edited by Hui C. Kimko and Stephen B. Duffull*
128. Affinity Capillary Electrophoresis in Pharmaceutics and Biopharmaceutics, *edited by Reinhard H. H. Neubert and Hans-Hermann Rüttinger*
129. Pharmaceutical Process Validation: An International Third Edition, Revised and Expanded, *edited by Robert A. Nash and Alfred H. Wachter*
130. Ophthalmic Drug Delivery Systems: Second Edition, Revised and Expanded, *edited by Ashim K. Mitra*
131. Pharmaceutical Gene Delivery Systems, *edited by Alain Rolland and Sean M. Sullivan*
132. Biomarkers in Clinical Drug Development, *edited by John C. Bloom and Robert A. Dean*
133. Pharmaceutical Extrusion Technology, *edited by Isaac Ghebre-Sellassie and Charles Martin*
134. Pharmaceutical Inhalation Aerosol Technology: Second Edition, Revised and Expanded, *edited by Anthony J. Hickey*
135. Pharmaceutical Statistics: Practical and Clinical Applications, Fourth Edition, *Sanford Bolton and Charles Bon*
136. Compliance Handbook for Pharmaceuticals, Medical Devices, and Biologics, *edited by Carmen Medina*
137. Freeze-Drying/Lyophilization of Pharmaceutical and Biological Products: Second Edition, Revised and Expanded, *edited by Louis Rey and Joan C. May*
138. Supercritical Fluid Technology for Drug Product Development, *edited by Peter York, Uday B. Kompella, and Boris Y. Shekunov*

ADDITIONAL VOLUMES IN PREPARATION

New Drug Approval Process: Fourth Edition, Accelerating Global Registrations, *edited by Richard A. Guarino*

Microbial Contamination Control in Parenteral Manufacturing, *edited by Kevin L. Williams*

New Drug Development: Regulatory Paradigms for Clinical Pharmacology and Biopharmaceutics, *edited by Chandrahas G. Sahajwalla*

Microbial Contamination Control in the Pharmaceutical Industry, *edited by Luis Jimenez*

Supercritical Fluid Technology for Drug Product Development

edited by

Peter York
University of Bradford
Bradford, United Kingdom

Uday B. Kompella
University of Nebraska
Omaha, Nebraska, U.S.A.

Boris Y. Shekunov
Ferro Corporation
Independence, Ohio, U.S.A.

MARCEL DEKKER, INC. NEW YORK • BASEL

critical-fluid extraction and high-performance liquid chromatography. J Chromatogr A 2000; 870:199–205.
160. Wang SP, Chen WJ. Determination of *p*-aminobenzoates and cinnamate in cosmetic matrix by supercritical-fluid extraction and micellar electrokinetic capillary chromatography. Anal Chim Acta 2000; 416:157–167.
161. Scalia S, Simeoni S. Assay of xanthene dyes in lipsticks by inverse supercritical fluid extraction and HPLC. Chromatographia 2001; 53:490–494.
162. Li JJ. Quantitative analysis of cosmetic waxes by using supercritical-fluid extraction(SFE)/supercritical-fluid chromatography (SFC) and multivariate data analysis. Chemom Intell Lab Syst 1999; 45:385–395.
163. Tena MT, Luque de Castro MD, Valcárcel M. Improved supercritical fluid extraction of sulphonamides. Chromatographia 1995; 40:197–203.

14

Development and Potential of Critical Fluid Technology in the Nutraceutical Industry

Jerry W. King
Los Alamos National Laboratory, Los Alamos, New Mexico, U.S.A.

Nutraceuticals, as the name suggests, are ingested substances which combine the benefit of food nutritional requirements while offering some aspect of therapeutic protection to the human body. Such foods and natural substances are called functional foods, designer foods, pharma foods, as well as many less elegant descriptors. Functional foods are similar in appearance to conventional foods, are consumed as part of a normal diet regime, and have demonstrated physiological benefit (i.e., reducing the risk of a disease state). Naturally derived products are purchased to enhance stamina and energy, for weight control, to avoid illness, and to compensate for the lack of exercise. Depending on the definition of a nutraceutical, the market ranges of such products is conservatively estimated to be US$3.15–4.6 billion in the United States and range from US$1.05–1.6 billion in Europe. A broader definition of "functional" food pegs their U.S. market value between US$14.2 and 17.6 billion, and if one assumes that 50% of the food selected for consumption is based on health or medical considerations, then the estimated value of the nutraceutical market expands to US$250 billion (1).

Consumers of nutraceuticals have expressed concern about pesticide or chemical residues, processing technology that contributes to ecological pollution, antibiotics or growth hormones in their foods, and the extensive use of preservatives in the foods they consume. It is for these reasons that technologies incorporating the use of critical fluids become important in the production of nutraceutical ingredients. Critical fluids, such as carbon dioxide (SC-CO_2), SC-CO_2/ethanol mixtures, and subcritical water, are environmentally benign processing agents; leaving no solvent residues in the final products while minimizing the oxidation or degradation of thermally labile components. Even a cursory inspection of the common classes of nutraceutical agents (herbs, specialty oils, plant extracts, specific protein fractions, and antioxidants) suggest a link between the two fields.

Table 1 lists some of the common and popular nutraceutical agents in use today, their application, as well as the use of critical fluid processing for their production. It should be noted that all of the nutraceuticals listed in Table 1 can be processed using critical fluids, however, a "yes" indicates that the actual production of the nutraceutical components has commenced. Indeed, a segment of the production capacity of the over 50 critical fluid processing plants worldwide are devoted to producing products for the nutraceutical market.

TABLE 1 Nutraceuticals and Their Therapeutic Use and Current Production via Critical Fluids

Nutraceutical	Utility	Processed via critical fluids
Saw palmetto	Prostate	Yes
Kava-kava	Anxiolytic	No
Hawthorne	Cardiotonic	No
Ginseng	Tonic	Yes
Garlic	Circulatory	Yes
Ginko biloba	Cognitive	No
St. John's wort	Depression	No
Chamomile	Dermatological	Yes
Echinacea	Colds/flu	Yes
Black cohosh	Gynecological	No
Lutein	Macular degeneration	Yes
Flavanoids	Anticancer	No
Isoflavones	Premenstral syndrome, circulatory	No
Marine oil fatty acids	Circulatory	Yes
Evening primrose	Inflammation	Yes
Phytosterols	Circulatory	Yes
Tocopherols	Antioxidant	Yes
Phospholipids	Cognitive	Yes

Critical fluid processing can be used in several modes for producing nutraceutical ingredients or functional foods. Exhaustive extraction in which SC-CO_2 or a SC-CO_2–cosolvent mixture is used to yield an extract equivalent to those obtained with organic solvent extraction or via pressing/expeling technologies (2), as documented in the recent literature (3). Fractional extraction in which the extraction pressure, temperature, time, or the addition of a cosolvent is varied on an incremental basis is also capable of producing extracts that are somewhat either enriched or depleted in the desired nutraceutical agent (4). Such fluid density-based or cosolvent-assisted extractions frequently yield extracts with considerable extraneous material; indeed, specifically extracting or enriching a desired solute out of a natural product matrix is somewhat akin to "finding a needle in a haystack." The problem is shown in Table 2, where many of the listed naturally occurring oils have been extracted with SC-CO_2; however, the targeted "nutraceutical" components in the right column occur in these SC-CO_2 extracts at very low concentration levels.

To enrich the concentration of desired component(s), researchers have resorted to fractionation techniques utilizing critical fluids. One of the simplest techniques separates the extract with the aid of multiple separators

TABLE 2 Natural Oils Extracted with Critical Fluids and Their Nutraceutical Components

Natural oils	Nutraceutical component
Rice bran	n-6, n-3 Fatty acids
Safflower	Phystosterols
Marine	Tocopherols
Sesame	Carotenoids
γ-Linolemic acid-enriched	Phospholipids
Oat	Tocotrienols
Almond	Oryzanol
Wheat germ	Sesamolin
Amaranth	Glycolipids
Essential	Conjugated fatty acids
Avocado	Lipoproteins
Grape seed	
Macadamia nut	
Kiwi	
Genetically modified oils	

Note: The nutraceutical components listed on the same line as a particular oil does not mean that nutraceutical moiety is associated with that oil.

placed downstream after the main extraction vessel. These separator vessels are held at different combinations of temperatures and pressures (5). Using such an approach, the fractionation of essential oils from waxes and oleoresins can be accomplished. The use of fractionation columns in which a temperature gradient is imposed on a solute-laden flowing stream of SC-CO_2, either in a batch or countercurrent mode, can also be used to fractionate supercritical fluid extracts. This technique has been used for the production of fish oil concentrates (6), fractionation of peel oil components (7), and mixed-glyceride fractionation (8). The coupling of critical fluids with chromatography on a preparative or production scale offers another alternative route to producing nutraceutical-enriched extracts. These chromatographic-based separations range from simple displacement or elution chromatographic schemes [i.e., for the removal of cholesterol (9)] to the more sophisticated simulated moving bed technology (10), the latter technique perhaps more favored for the purification of pharmacological compounds.

Although there are many examples for processing nutraceutical ingredients using critical fluids, we will discuss here specific methods for producing extracts, fractions enriched in nutraceutical ingredients (i.e., products containing sterols and sterol esters from natural sources). Sterol esters have been clinically evaluated (11) and proven to effective for inhibiting cholesterol absorption and synthesis in the human body. This has resulted in the marketing of two commercial products, Benecol and Take Control, on a worldwide basis (12). Likewise, nutraceuticals containing tocopherols or phospholipids can be readily produced via critical fluid technology, as will be described shortly. In addition, an alternative route for producing "naturally" synthesized nutraceutical components, such as the above sterol esters, or transesterified esters can be accomplished in SC-CO_2, particularly if enzyme catalysts are used. Also amenable to this type of synthesis are the "omega-3" fish oil type of esters, which are highly valued and an article of commerce in the nurtraceutical field.

1. THE RATIONALE FOR USING CRITICAL FLUIDS

The use of critical fluids for the extraction and refining of components in natural products has now been facilitated for over 30 years. Early success in the decaffeination of coffee beans and isolation of specific fractions from hops for flavoring beer, using either supercritical carbon or liquid carbon dioxide, are but two examples of the commercial application of this versatile technology. Critical fluid technology, a term that will be used here to embrace an array of fluids under pressure, has seen new and varied applications which include the areas of engineering-scale processing, analytical, and materials modification.

However, beginning in the early 1990s, an awareness of the potential of critical fluid processing as a viable component of the newly coined term "green" processing arose (13). This, coupled with an increasing consumer awareness of the identity and use of nongreen chemical agents in food and natural products processing, suggested a promising future for the use of such natural solvents as supercritical fluid carbon dioxide (SC-CO_2), ethanol, and water. Indeed, labels on food products and ingredients which tout, "naturally decaffeinated" and "nature in its most concentrated form: high pressure extraction with carbon dioxide," have an appeal to the consumer who is aware of food safety issues. Recently, certain nutraceutical products have been labeled as "hexane-free" to alert the general public to the undesirable use of organic chemical-based processing agents.

There is a certain synergy that exists between critical fluid processing and the use of nutraceuticals. Consumer use and acceptance of these natural products is heightened by the appeal that they have been "naturally processed." However, this is not a new development and it is interesting to note that many of today's nutraceutical components have already been extracted or separated using the above-named natural agents. In addition, critical fluid processing has also been used to create healthier and functional foods, such as eggs with a reduced cholesterol content (14), low-fat nut products (15), pesticide-free natural products (16), spice extracts (17), as well as cholesterol reduction in meats (18). There is no doubt that the development of such products coupled with a proper commercial marketing campaign provides a powerful stimulus for the consumer to try these products.

2. A GREEN PROCESSING PLATFORM

As noted previously, SC-CO_2 reigns supreme as the principle processing agent in critical fluid technology. However, this situation is changing, particularly with the recognition that SC-CO_2 cannot be effectively utilized for all tasks and is a relative poor extraction solvent for polar compounds. For certain applications, the addition of a minimal amount of cosolvent [usually an organic solvent having a higher critical temperature (T_c) than CO_2] suffices to improve the extraction of specific components from a natural product matrix. However, the number of GRAS (Generally Recognized As Safe) cosolvents is rather limited (e.g., ethanol, acetic acid). Such cosolvents can be used in conjunction with SC-CO_2 under conditions which can favor the formation of a one-phase or multiphase extraction medium, capable of producing the desired end result.

Other processing media which embrace the green processing concept are the use of liquefied gases (e.g., LCO_2) and certain liquids under pressure above their boiling point. By the application of external pressure, liquids such as water and ethanol can be used above their boiling point. Such liquids can be used to produce equivalent or superior products compared to those derived by using conventional liquid solvent extraction (19). For example, St. John's wort's active components can be more effectively isolated using critical fluids and/or by using SC-CO_2 along with subcritical water (20). Recently, the author and his colleagues have utilized subcritical water between 120°C and 160°C to isolate anthocyanins from natural berry substrates (21). In the processing of natural products, optimization of the extraction temperature is important in order to avoid degradation of the extracted components, such as the anthocyanins. For the extraction of anthocyanins, 120°C proves to be the optimal temperature. LCO_2 has also been used at near-ambient or subambient temperatures (22,23), thereby avoiding conditions conducive to thermal degradation of the extracted components.

Subcritical water complements SC-CO_2 for the environmentally benign processing of nutraceuticals. Its phase diagram (Fig. 1) is similar to that of CO_2, although the critical temperature and pressure of water are much higher (T_c = 374°C, P_c = 221 atm) than those for CO_2. However, the region in the phase diagram that is of interest for processing nutraceuticals lies between 100°C and 200°C in the liquid region defined in Figure 1 and at pressures less than 100 atm. Recent studies have shown that subcritical water under these conditions can be effective for the extraction of the natural products, such as cloves (24) and rosemary (25).

Considerable literature exists on the properties and use of water in the superheated state, and its temperature has been shown to be a key parameter

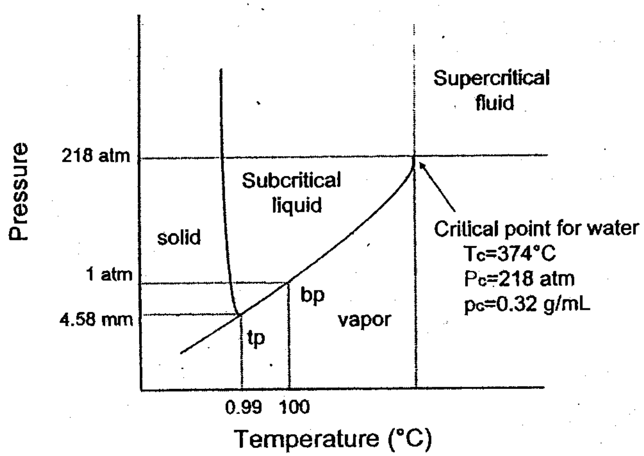

FIGURE 1 Phase diagram of water.

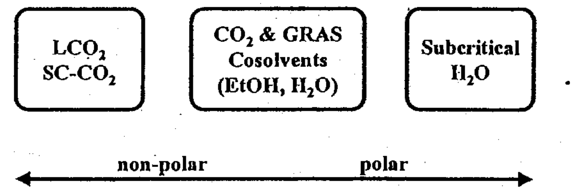

FIGURE 2 "Green" critical fluid processing options.

for regulating the solvent power of superheated water (26). The dielectric constant of water varies inversely with temperature; it varies from 30 to 60 over a temperature range of 100–200°C. Dielectric constants of this magnitude are in the range of those exhibited by polar organic liquids. Therefore, subcritical water can be potentially used as a substitute for less desirable organic solvents, including ethanol, to extract and process natural products.

Figure 2 summarizes the above in terms of offering an "all-natural" approach to processing natural products for nutraceutical ingredients. On one end of the solvent scale lies SC-CO_2 and LCO_2, whereas pressurized water on the other end is available for isolating polar moieties. Several combinations of GRAS cosolvents can be coupled with CO_2 for cases in which this approach proves viable. Sequential processing of a natural substrate by the use of these various fluids is also possible, as suggested by the hypothetical scheme noted in Figure 3 for soybeans as a natural substrate. Here, the nonpolar components, such as carotenoids, triterpenes, or phytosterols, are preferentially removed by CO_2 followed by extraction with a CO_2–cosolvent combination that can remove the more polar components, such as phenolic

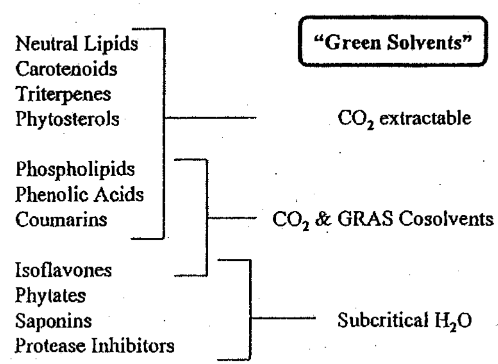

FIGURE 3 Separation scheme for nutraceutical components in soybeans.

acids or coumarins. Finally, after removal of the above components, subcritical water can be applied to isolate the isoflavones, phytates, and so forth. It should be recognized that some of targeted nutraceutical compounds may occur in each of these "green" solvent combinations. An additional benefit of the process depicted in Figure 3 is that left-over residual proteinaceous meal is available for further use, devoid of any solvent residues. This is an appealing extraction and/or fractionation scheme that can be accomplished using the same high pressure processing equipment, as has been noted by King (27).

3. TECHNIQUES FOR ENRICHING AND ISOLATING NUTRACEUTICALS

The development of critical-fluid-based techniques for isolating nutraceutical ingredients requires sequential development in steps of increasing complexity and scale-up. These are summarized as follows:

Bench-scale supercritical fluid extraction (SFE) evaluation of process feasibility
Establishment of the need for fractionation
Pilot-plant evaluation
Scale-up to plant Stage

Bench-scale evaluation of the feasibility of extracting a nutraceutical is usually accomplished with the aid of a small-scale extractor. Such units are available commercially, but an increased experimental flexibility is achieved by constructing a customized SFE unit. Alternatively, there exists the availability of small-scale SFE equipment, normally intended for analytical uses of critical fluid technology, which can also be used to optimize and assess the extraction of a natural product (28). Both of the above approaches can also incorporate the introduction of a cosolvent into the critical fluid, should this be required in the processing of a nutraceutical source.

Enrichment of a nutraceutical ingredient to a sufficient purity or concentration cannot always be accomplished by using just an extraction step, and this will necessitate the use of a fractionation method, in one of several forms, or even by combining two or more fractionation methods. A combination of mixer/settler modules is one mode of amplifying the effects of single-stage SFE (29). A more popular approach for fractionating natural products is the use of a fractionating tower with internal packing, and that frequently will incorporate a temperature gradient along its length (30). Introduction of the material to be fractionated can be made at the bottom, top, or an intermittent position in the column. Both cocurrent and countercurrent operational modes can be enacted with respect to contacting the critical fluid with the substrate whose components need to be separated.

Chromatographic fractionation has also been utilized (31), including the use of simulated moving-bed technology (32). Examples of these chromatographic options will be discussed later.

The pilot-plant evaluation stage incorporates both a scale-up of the previously described approaches as well several other processing options. Although somewhat rare, single-pass (with respect to the extraction fluid) pilot and production plants are known to exist, but plants designed for fluid recycling with integrated heat exchangers are much more the norm. Batch pilot- and production-scale plants have been in use for some time, and several novel approaches have been described which allow the continuous feed of substrates into extraction or fractionation units (i.e., lock hoppers, augers, etc.) (33).

Final scale-up to the production plant stage is a serious undertaking, with respect to mechanical complexity, safety, and economics. Such plants have traditionally been more or permanent in design; however, there is a trend toward increased flexibility (e.g., multiple-use modules for extraction, fractionation, reaction), considering the initial investment in the in common pumps and fluid sources. Portable extraction units have also been constructed and their feasibility demonstrated for the field side processing, thereby permitting extraction of natural products immediately after they are harvested. This avoids degradation during the transport of the nutraceutical ingredient, (i.e., medicinal ingredients in chamomile).

3.1. Examples of Experimental/Processing Equipment and Methods

To describe some of generic approaches mentioned in the previous section for processing nutraceuticals, several examples are provided in this subsection to illustrate the equipment which is appropriate for conducting extractions and fractionations with critical fluids. Figure 4 shows a schematic of a benchtop extraction system that has proven to be very versatile in our laboratories. A fluid source, A (e.g., CO_2), which can be can be either gaseous or liquefied, is, of course, required. Use of liquefied CO_2 has the advantage of being more compatible because this is what is found most frequently in use on scaled-up equipment. A compressor or liquid pump, C, delivers the fluid through a tandem switching valve, SV-1 and SV-2, to a tubular extraction vessel cell that is held at a constant temperature to maintain the fluid in its desired critical state (subcritical or supercritical). The fluid then passes over the material in the extraction vessel and is routed through another switching valve arrangement. In Figure 4, either a micrometering valve, MV, or back-pressure regulator, is used to reduce the pressure on the fluid as it exits the extraction/fractionation stage.

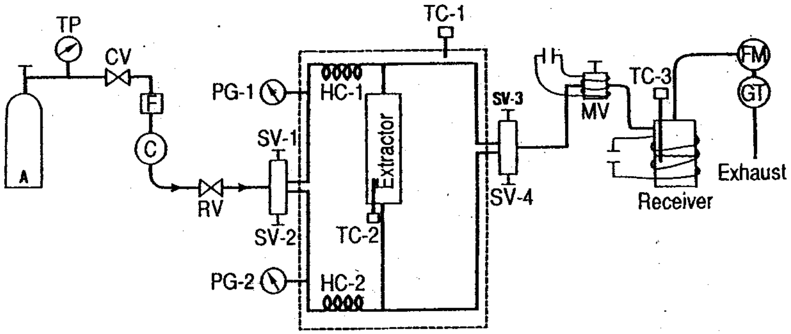

FIGURE 4 Bench-scale supercritical fluid extraction system.

The receiver vessel can be of several formats but will frequently be packed with internal packing to eliminate entrainment of the extracted components forming in the rapidly expanding critical fluid. Flow conditions under ambient conditions are measured with the aid of a flow meter, FM, and fluid totalizing module, GT. This described SFE unit can be changed to accommodate fractionation and reaction experiments as has been shown and documented in our laboratories at a modest cost (34). Such equipment today exists commercially but may be more limited with respect to pressure range, fluid flow rate, and size of the extraction chamber (34).

An increase in processing capability to a mini-pilot-plant stage is shown in Figure 5. This unit incorporates a fluid recycle option which reduces substantially the mass of extraction fluid required for SFE, because the initial charge of extraction fluid is used continuously throughout the extraction. The 4-L extraction vessel permits multikilogram quantities of natural product to be extracted. For this vessel, and others utilized for conducting extractions up 70 MPa (680 atm), a safety factor of 2.5 times the maximum operational pressure is specified when ordering these vessels. Back-pressure or metering valve arrangements used for pressure reduction of the extraction fluid are heated to overcome the attendant Joule–Thomson cooling effect as the depressurized fluid is jettisoned to the collection vessel.

The collection vessel must be of sufficient size to allow separation of the extracted solutes from the depressurized fluid. For these purposes, a 2-L collection vessel is utilized, as shown in Figure 5. By following the vector arrows in Figure 5, one can trace the depressurized fluid flow path back to the compressor, where the fluid is repressurized to continue the extraction. A sorbent-laden column may be inserted in the low pressure side of this scheme for purifying the exhausted fluid of any unwanted odoriferous volatile com-

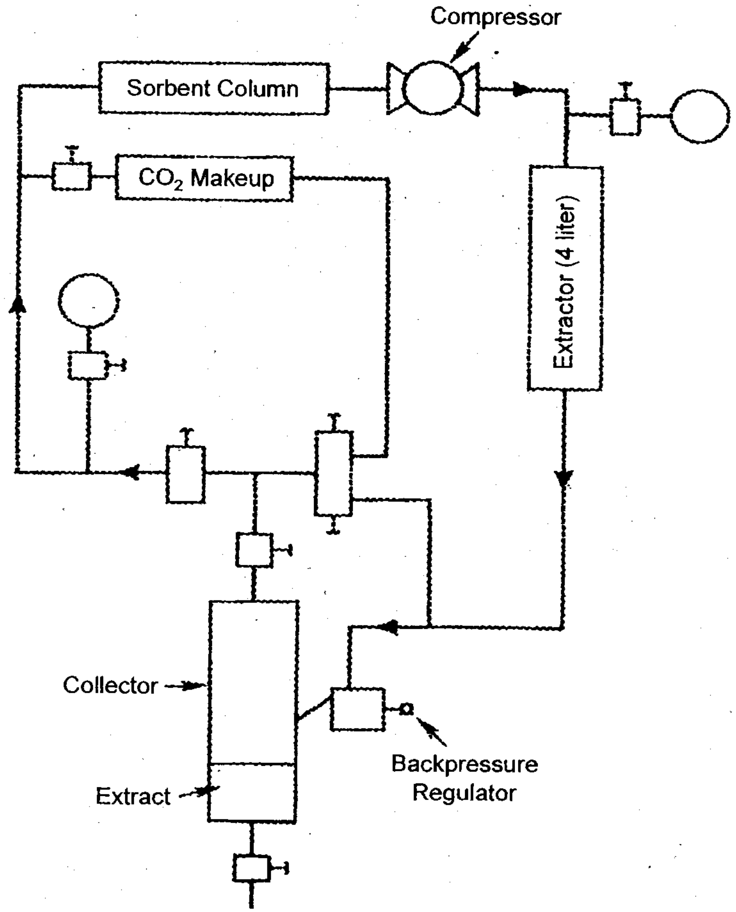

FIGURE 5 Continuous recycle supercritical fluid extractor.

pounds. A provision for makeup of fluid lost upon draining the extract from the receiver vessel is noted in Figure 5 as the "CO_2 makeup."

A larger semicontinuous pilot plant (35) is shown in Figure 6. It consists of three, 4-L extraction vessels, arranged with an appropriate valve sequence to permit several modes of operation. However the principle feature that distinguishes it from the smaller unit noted in Figure 5, is that it allows for semicontinuous processing using the tandem vessel arrangement depicted in Figure 6. Here, carbon dioxide, the extraction fluid, can be routed sequential to one or more of the extraction vessels, which can be operated so that one of

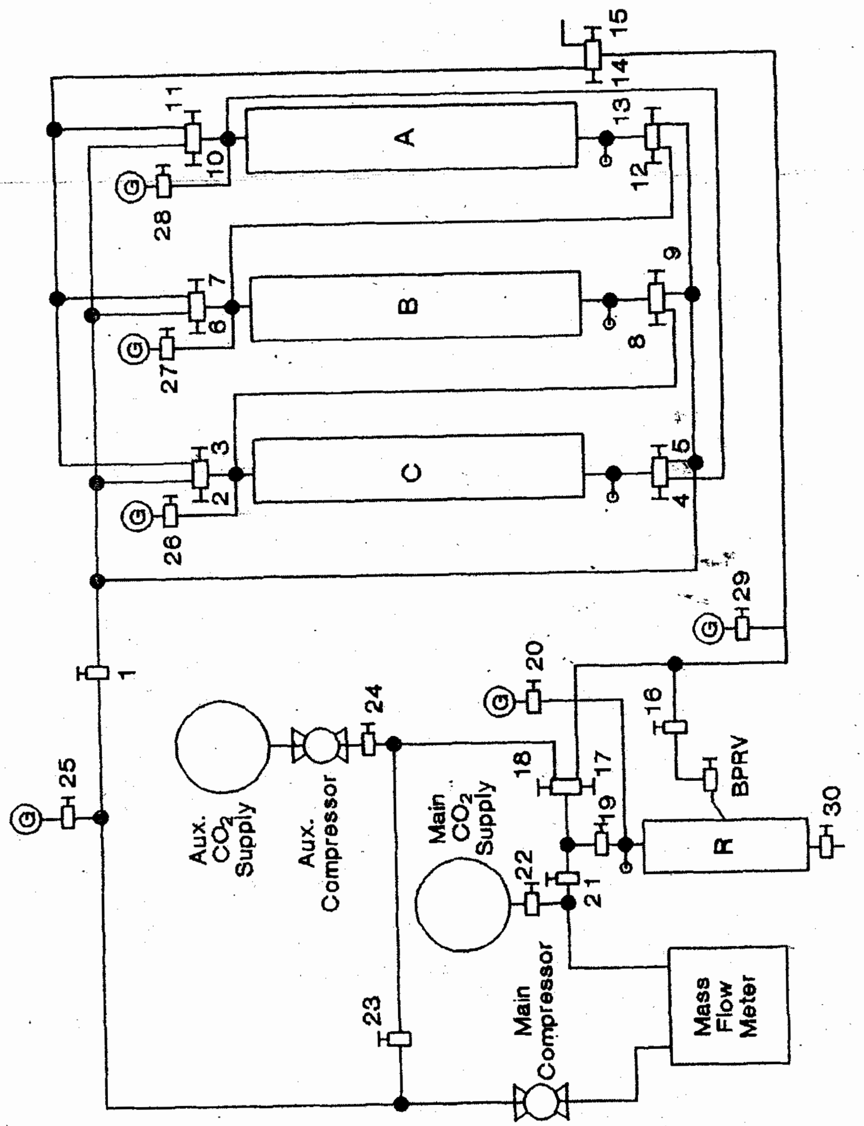

FIGURE 6 Semicontinuous pilot-plant extraction system.

the vessels, A, is being extracted while another vessel, B, is being loaded with product to be processed, and a third vessel, C, is capable of undergoing pressurization/depressurization. That these operations can be accomplished in parallel is also apparent, lending the unit to semicontinuous operation, even for the processing of granular solids.

Other types of pilot plant, including commercial units embracing this principle, are available. Some selected vendors of pilot plants, although this is not an exclusive list, include UHDE, Thar Designs, Applied Separations, Chematur, and Separex.. Likewise, bench-scale equipment for preliminary evaluations are manufactured by such companies as Autoclave Engineers (now called Snap-Tite), Chematur, Nova Swiss, Applied Separations, Nova Sep, Thar Designs, Pressure Products, Inc., Supercritical Fluid Technologies, and Separex.

In the future, extraction and other processing options may be accomplished with the aid of an expeller; that is, the critical fluid component will be introduced into the expeller barrel. This mode of processing not only allows extraction/fractionation to be accomplished on a continuous feed of raw material but also allows the introduction of nutraceutical ingredients through selective dissolution in the fluid, permitting them to be naturally "impregnated" into a product matrix. The addition of CO_2 into the barrel of the extruder, where it becomes a supercritical fluid due to the heat and pressure generated during the extrusion process, not only facilitates solubilization of materials from the substrate being processed but also enhances the fluidity of the potential extract (e.g., a nutraceutical-based oil). Two active companies in this field are Critical Processes Ltd. in North Yorkshire, England and Crown Iron Works in Minneapolis. The former firm is focused on nutraceutical extraction, citrus oil deterpenation, and so forth and the latter company is concerned with CO_2-assisted recovery of oils from seeds.

3.2. Fractionating with Critical Fluids

The ability to fractionate naturally derived materials in a benign way using critical fluids is of particular interest to processors of nutraceutical ingredients. This is due to the fact that to obtain useful extracts, an enrichment or isolation of the more highly purified form of the nutraceutical component increases the value and utility value of the derived product to nutraceutical manufacturers. Hence, approaches to achieve the above result are discussed in this subsection.

One of the important methods of critical fluid fractionation involves the countercurrent separation of phospholipids from a vegetable oil. A system to achieve this end is presented in Figure 7. Here, high pressure CO_2 is fed into a pressure vessel packed with segmented gauze mesh packing (the "refining

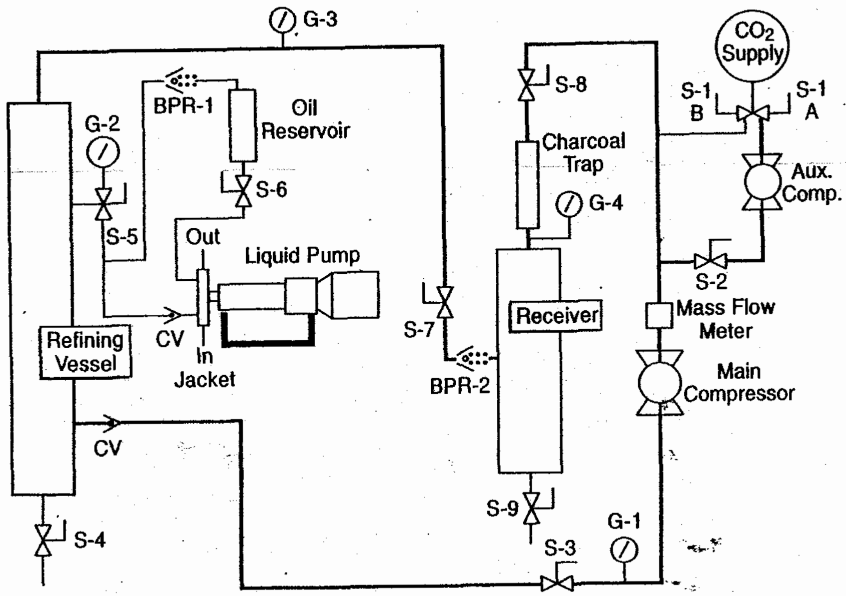

FIGURE 7 Continuous countercurrent refining system.

vessel" in Fig. 7), where it travels upward, contacting soybean oil, which is pumped into the top of the refining vessel using the designated liquid pump. The two media contact one another in the refining vessel, in which oil is solubilized in the SC-CO_2, and the phospholipids being insoluble as the CO_2 descends to the bottom of the refining vessel. The oil can be recovered by lowering the pressure and temperature in the receiver vessel, allowing recycling of the CO_2 back to the main compressor. By using this technique, an extract enriched in lecithin precipitate can be isolated without the use of organic solvents (36).

A somewhat more sophisticated fractionation method involving the use of vertical packed fractionating towers is currently being applied to enrich nutraceutical ingredients from liquid natural feedstocks. The liquid to be fractionated can either be fed concurrently or countercurrently into the fractionating column. There are certain advantages in terms of fractionation efficiency that are provided when using the countercurrent mode and by introducing the liquid feed into the center of column, thus creating extraction and raffination sections. An example of a fractionating column operated in the concurrent mode is shown in Figure 8. For experiments conducted in the author's laboratory, the unit is usually operated in a batch mode, but it can be

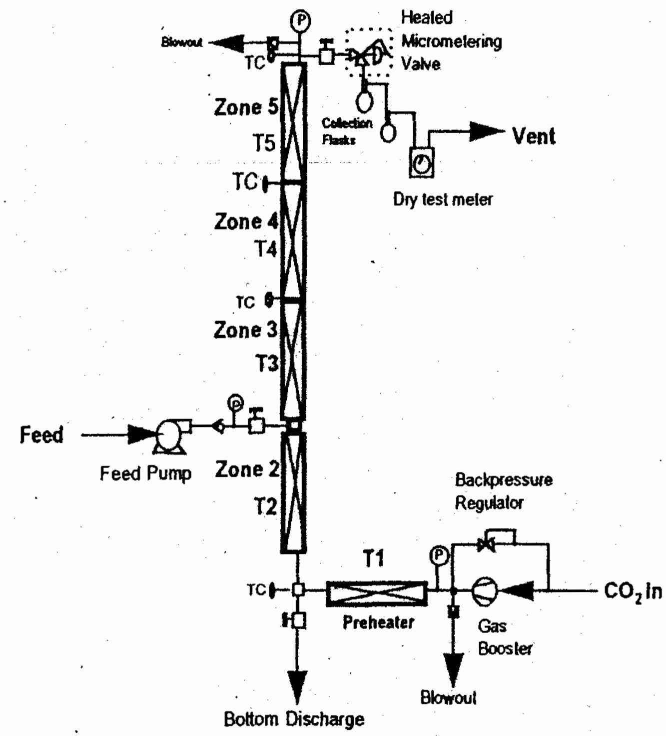

FIGURE 8 Thermal gradient supercritical fluid fractionation column.

made to operate on a semicontinuous basis, using a liquid pump to feed substrate into the column (see in Fig. 8). The components to be separated in the substrate are subjected to a thermal gradient along the length of the fractionating tower, where each of the designated sections of the column have an increasing temperature for the sequence T2, T3, T4, and T5. This allows fractionation of the components in the substrate feed based not only on their relative solubilities in the decreasing CO_2 density gradient but also according to the increase in their respective increasing vapor pressures as they ascend the column. This type of fractionation system has been used to deacidify olive oil, to deterpenate citrus oils, and to fractionate fish oils or butter fat. The recent approach of Clifford et al. (24) involving the deterpenation of citrus oils using subcritical water also applies this fractionation principle.

Chromatographic fractionation using critical fluids as mobile phases has been studied for some time now; however, scaleup from the analytical regime has been less prevalent. Studies at the National Centre for Agricultural

Utilization Research by King and co-workers (31) have demonstrated that preparative supercritical fluid chromatography (SFC) can be coupled advantageously with a selective SFE enrichment stage to yield concentrates rich in nutraceutical ingredients. As shown in the processing scheme in Figure 9, flaked soybeans are initially extracted at a relative low pressure to enrich the components of interest, the tocopherols. This fraction is then moved sequentially on to a sorbent-filled column for further fractionation to yield a tocopherol-enriched extract of nutraceutical value. The advantage of this approach is it allows one to enrich a particularly valuable nutraceutical ingredient from a natural matrix, without contaminating of the remaining matrix with a noxious agent. Enrichment factors relative to the tocopherol content in the original soybean flakes are tabulated in Table 3. Note that these are only modest tocopherol-enrichment factors for application of the single SFE step; however, significant enrichment of the desired components can be obtained by then applying SFC for further fractionation and enrichment of the tocopherols (Table 3).

Recently, using a similar approach, Taylor et al. (37) have been able to produce concentrates enriched in phospholipids (PPLs) for potential use in the nutraceutical industry. Table 4 summarizes the relative amounts of PPLs via initial SFE isolation and in the fractions obtained after SFC. Here, the major component in the starting substrate (soybean oil triglycerides) was initially reduced in the SFE step using neat SC-CO_2, followed by sequential SFE–SFC utilizing SC-CO_2–cosolvent mixtures on the lecithin-containing residue which remains after SC-CO_2 extraction.. By using SC-CO_2/ethanol/water fluid phases, one not only could perform preparative SFC for enriching the PPLs but can also obtain, in certain cases, individual purified PPL moieties. This is achieved by selective density and compositional programming of the SFC fluid phase, coupled with time-based collection of eluent fractions.

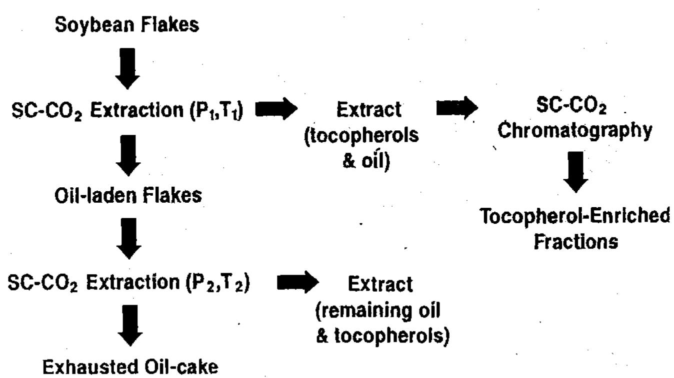

FIGURE 9 Tocopherol-enrichment/fractionation by the SFE–SFC technique.

TABLE 3 Enrichment Factors of Tocopherols from Soybeans by SFE and SFE–SFC

Tocopherol	SFE	SFE–SFC
Alpha	4.33	12.1
Beta	1.83	2.4
Gamma	3.94	15.0
Delta	3.75	30.8

Previously, mention has been made of the use of subcritical water as a "green" processing agent. Studies performed in the 1970s by Schultz and Randall (38) showed that useful fractionations of flavor components could be obtained by using LCO_2 in a countercurrent mode with fruit-based aqueous feedstocks. This approach produced fruit flavor essence concentrates enriched in the more hydrophobic components. Recently, a similar method exploiting LCO_2/aqueous phase partitioning has been developed by Robinson and Sims (39) using a microporous membrane to further increase the fractionation efficiency in such systems. This Porocrit fluid fractionation process is depicted in Figure 10 and shows "near"-critical CO_2 being fed into a concentric tube arrangement, countercurrent to the flow of aqueous liquid feedstock, which is being pumped internally through a microporous membrane. Again, an enriched extract exits with the depressurized CO_2, producing an aqueous fruit aroma of the composition described in Table 5. Here, the listed components in the feedstock have been concentrated in many cases, over 100-fold with respect to the starting concentrations. Note also that the

TABLE 4 Relative Amount of PPLs from Soybeans in SFE Isolates and in SFC-Collected Fractions

Phospholipid	SFE[a]	SFC
Phosphatidylethanolamine	16.1	76.8
Phosphatidylinositol	9.2	74.9
Phosphatidic acid	2.8	20.8
Phosphatidylcholine	15.6	55.8

Note: All data in percent of that component.
[a] Relative to other eluting constitutents (oil and unidentified peaks).

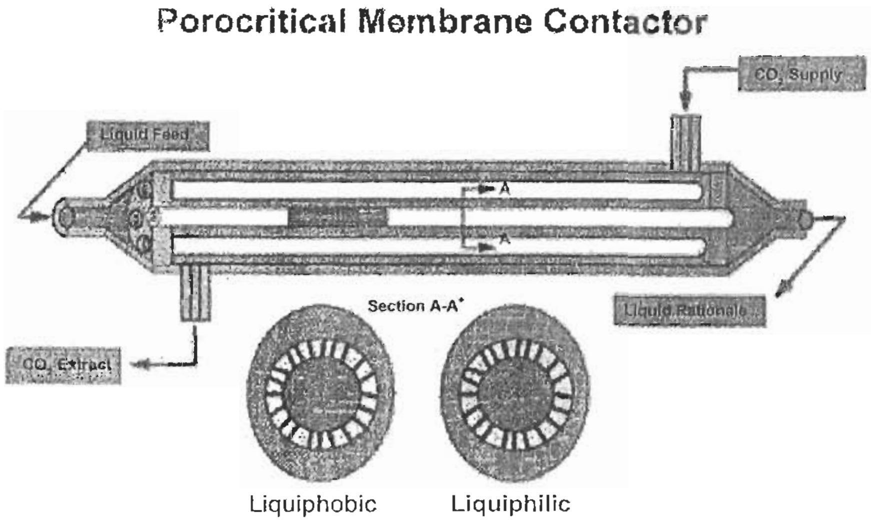

FIGURE 10 Porocrit continuous fluid extraction process.

TABLE 5 Enrichment of Aroma Constituents by the Porocrit Process

Compound	ppm in feed	Conc. factor in extract	% Depletion
Methanol	4,220	3	---
Ethanol	105,000	7	20
1-Propanol	270	82	69
Amyl Alcohol	17	115	81
Hexanol	3	109	95+
Octanol	5	110	95+
Z-3-Hexanol	10	127	95+
α-Terpinenol	10	98	95+
Terpinen-4-ol	4	106	95+
Ethyl acetate	29	106	85
Ethyl butyrate	23	106	88
Acetal	37	147	39
ε-2-Hexenal	19	132	86
Hexanal	10	100	84
Octanal	5	156	95+
Citronellal	3	62	95+

Porocrit process is very effective at removing the flavor components, as judged by the listed solute depletion data given in the last column of Table 5.

4. ROLE OF ANALYTICAL SCF TECHNOLOGY IN SUPPORT OF NUTRACEUTICALS

The analytical use of critical fluids spans over three decades of endeavor and applications (40,41) and there are many excellent tomes describing activity in this field (42,43). It is not the intention of this review to provide a detailed description of the use of analytical methodology for characterizing nutraceutical products, although such techniques as SFC, can rapidly provide valuable information for the chemist and product formulator (44,45).

There is no doubt that analytical SFC is a logical choice to characterize extracts or fractions obtained by SFE and fractionation, but perhaps of more importance is to describe options that exist for utilizing such analytical instrumentation to assist in optimizing and developing processing methods to produce nutraceuticals for the marketplace. A summary of the information obtainable using analytical-scale techniques and methodology are given in Table 6.

With the advent of automated analytical SFE equipment, it has become possible to rapidly ascertain what extraction or fractionation conditions would be most relevant in scaling up the process. In the United States, analytical SFE instrumentation is produced by such firms as Isco, Applied Separations, Leco, and Jasco. In Europe, analytical-scale SFC equipment is available from Berger Instruments, Thar Designs, Jasco, and Sensar. The equipment is obtained from these vendors can be, if needed, slightly modified to study the conditions that are amenable to processing nutraceuticals. King (34) has provided a interesting review of how lab-constructed equipment can be used for both analytical and process development purposes.

Two examples will be cited that show how analytical SFE instrumentation can be used to obtain information related to the isolation of nutraceu-

TABLE 6 Critical Fluid Analytical Technology—Relevance to Nutraceutical Product Development

To indicate solubility or extractability of a compound
For fractionating a natural product
In support of process development
For analysis of critical-fluid-derived extract
To deformulate a commercial product
To determine required physicochemical data

tical ingredients. Chandra and Nair (46) have studied the extraction of isoflavone components, such as daidzein and genistein, from soya-based products, using a manually operated SFE system. Neat SC-CO_2 proved relatively ineffective in removing the isoflavone components from the various soya-based matrices; however, by simply varying the conditions for effecting the extraction, it was found that 20 vol% ethanol in SC-CO_2 at 50°C and 600 atm could remove over 90% of the isoflavones in under 60 min extraction time. These survey extractions were performed on sample sizes ranging from 2 to 10 g, saving considerable time and labor in ascertaining what conditions were optimal for the extraction of these components. The need for relatively high pressures and cosolvent to remove the isoflavones from the matrix suggest that subcritical water extraction might be another alternative for isolating these nutraceutical components.

An automated SFE option has been utilized by Montanari et al. (47) and Taylor et al. (37,48) to develop the conditions most amenable to isolating PPL concentrates from deoiled soy meal. In the former case, a set of conditions was tested by programming the microprocessor controller of the automated SFE to run a large number of extractions on common samples at various pressures, temperatures, and cosolvent (ethanol) levels. The results showed that at 70°C and 40.7 MPa with 10 mol% ethanol, phosphatidylcholine could be extracted preferentially relative to the other phospholipids in the soya meal. By utilizing higher pressures, it was found that the total amount of phospholipids extracted could be substantially increased at the slight reduction of the phosphatidylcholine content in the final extract.

Similarly, an automated SFE unit was also used to ascertain what conditions were necessary to elute and separate phospholipid moieties from extracts obtained from SFE and other extraction methods (37,48). In this case, the PPL-containing extract was layered on top of an adsorbent, such as alumina or silica gel, and elution and separation of the target compounds were assessed by changing the pressure, temperature, quantity, and composition of the cosolvent in the supercritical fluid eluent. As summarized in Table 7, pure SC-CO_2 aided in removing the nonpolar (triglyceride) components from the deposited sample, but the presence of a binary cosolvent with the SC-CO_2 proved necessary to effect elution of the PPLs from the sorbent bed. By collecting various fractions during the stepwise elution from the column, while varying the eluent conditions, various phospholipids could be enriched as indicated in Table 7. These experiments could be run in several days and even overnight on the automated SFE module, saving considerable cost and effort before scaling up the SFE–SFC process to a preparative level. Using a similar approach, experiments were run on other natural product matrices in terms of ascertaining whether comminuting the sample prior to conducting a SFE was necessary. Alternatively, such instrumentation can assist in identifying what part of a natural plant actually contains the targeted components of interest (49).

TABLE 7 SFC Fractionation of Lecithin on Silica Gel

Fraction collected[a]	Eluent parameters	Predominate compounds
#1	350 bar, 50°C, CO_2	Triglyceride oil
#2	350 bar, 50°C, CO_2/M	Triglyceride oil
#3	350 bar, 50°C, CO_2/M	
#4	500 bar, 50°C, CO_2/M	Phosphatidylethanolamine
#5	500 bar, 80°C, CO_2/M	Phosphatidylinositol + phosphatidycholine
#6	500 bar, 80°C, CO_2/M	Phosphatidylcholine
#7	500 bar, 80°C, CO_2/M	Phosphatidylcholine

[a] The fraction #2 modifier is 10% ethanol : water (9 : 1). The fractions #3–7 modifier is 25% ethanol : water (9 : 1). M = modifier (cosolvent).

It was mentioned previously that analytical SFC could be of considerable use in evaluating the content of nutraceutical-containing extracts or feedstocks. This is particularly true if one is looking for a rapid analysis method for quality control of selected ingredients or to monitor qualitative differences in processing and raw materials. The elution order of lipophilic solutes is well known in capillary SFC (50) and permits the separation and identification of key lipophilic nutraceutical components. For example, in Figure 11, the high-resolution separation of fatty acids, squalene, tocopherols, phystosterols, and various glycerides contained in deodorizer distillate is shown using analytical capillary SFC using flame ionization detection (FID) (51). The resultant profile is of a valuable feedstock for nutraceutical components and indicates that its components are soluble in SC-CO_2 (the mobile phase for this analytical SFC separation), as well as revealing valuable information on the chemical composition of the sample to the analytical chemist. This is an excellent assay method considering that the sample preparation is minimal and the analysis time is under 45 min.

On the subject of sample preparation, analytical SFC can save the analyst considerable time, as illustrated by the SFC profile of the composition in a nutraceutical capsule containing sawtooth palmetto berry extract (Fig. 12). In this case, the extract was dissolved and diluted with a minimal amount of hexane and directly injected into the chromatograph. By density programming the CO_2 mobile phase, a high-resolution chromatogram can be facilitated. The complexity of the sawtooth palmetto berry extract is apparent and

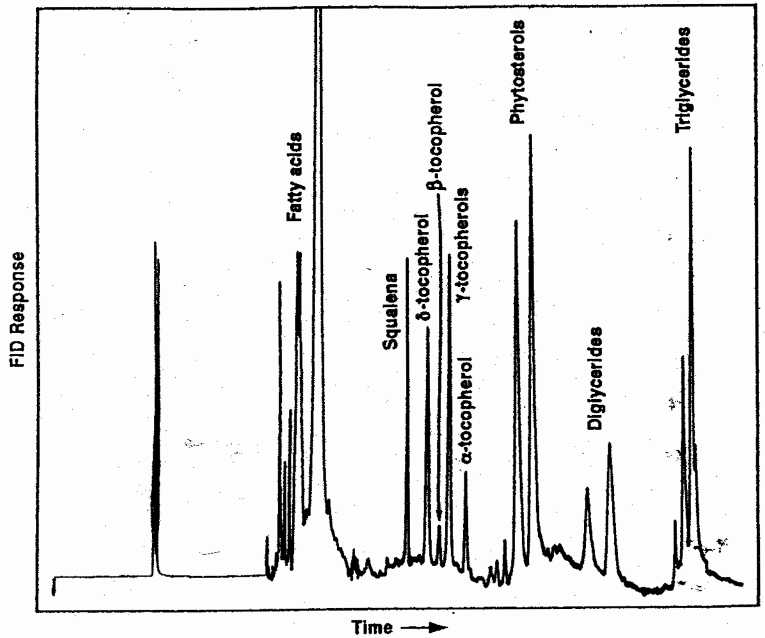

Figure 11 Capillary SFC characterization of deodorizer distillate feedstock.

consists of a mixed composition of fatty acids and trace sterol components. Recently, a similar approach has been used by others for characterizing saw palmetto extracts (52).

5. PRODUCTION PLANTS AND NUTRACEUTICAL PRODUCTS

The purpose of this section is to provide a brief overview of the magnitude and scope of critical fluid technology as applied to the production of industrial products, including nutraceuticals. Information on the use of critical fluid production facilities is be limited due to proprietary constraints; however, production processes using supercritical fluids have existed for over 30 years and critical fluid processing is no longer a novelty industry. These production facilities vary considerably in the magnitude of the operation, ranging from the large Houston-based decaffeination plant of General Foods to smaller extraction facilities focused on the production of noncommodity items. Aside

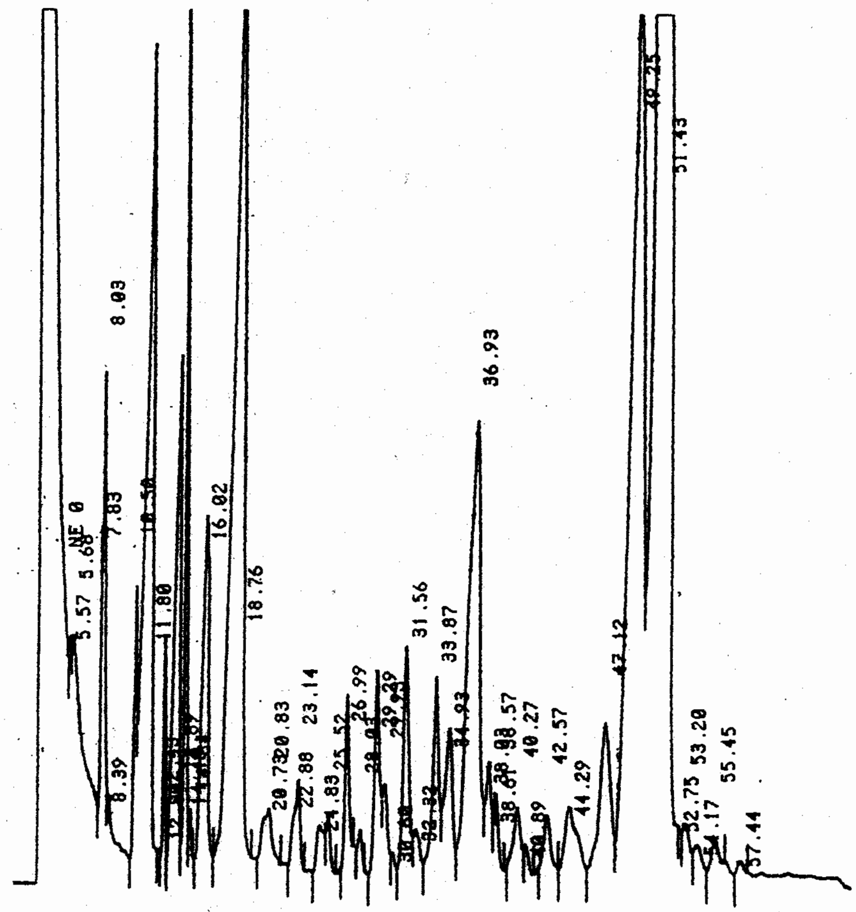

Figure 12 Capillary SFC analysis of capsule containing sawtooth palmetto berry extract.

from decaffeination plants, there is a sizable segment of the production facilities devoted to the processing of hops. However, these facilities are devoted to hops processing for only part of the year and potentially have additional capacity which could be devoted to processing of materials having nutraceutical value.

There are over 50 plants (not pilot plants) worldwide devoted to critical fluid processing. Currently, most of these are located in Germany, the United States, France, and Japan. Other nations, such as Britain, Australia, India, and Italy, continue to develop more capacity for critical fluid processing; other nations with a rich litany of natural products will undoubtedly enter the marketplace as users. Table 8 presents a list, which is not inclusive, of many users of critical fluid extraction for food and natural product processing throughout the world. Key players in identified market segments are as follows: decaffeination—General Foods, SKW Trostberg, Kaffee HAG, Hermsen; hops processing—HVG Barth (NATECO$_2$), John Haas, Yakima Chief, Carlton United Breweries, Steiner Hops, English Hops, SKW Trostberg; flavors/spices—Cultor, Quest, Flavex, Norac, US Nutraceuticals, Ogawa, Fuji Flavor, Kobe, Mori Oil Mills, Takeda. Many of the processors listed under the generic heading of flavors/spices process a variety of other natural products such as specialized oils, natural pigments (e.g., Flavex is involved in the processing of ginseng among other moieties). As noted earlier, many of these companies have additional capacity for fulfilling the needs of the nutraceutical market and other firms, on a more diminutive scale, are preparing to enter the market focusing on the processing of niche natural products. Some of the new companies addressing nutraceuticals processing specifically are US Nutraceuticals, KD–Pharma–IQA, Wells Investments Ltd., Aromtech OY, GreenTek 21, and Arkopharma.

TABLE 8 Organizations Processing or Offering Critical Fluid-Derived Products

Flavex (Germany)	Fuji Flavor (Japan)
Hermsen (Germany)	Kobe (Japan)
HVG Barth (Germany)	Mori Oil Mills (Japan)
Kaffe HAG (Germany)	Ogawa (Japan)
SKW Trostberg (Germany)	Takasago (Japan)
KD–Pharma–IQA (Germany–Spain)	Takeda (Japan)
General Foods (United States)	Cultor (France)
John Haas (United States)	HITEX/Separex (France)
Praxair (United States)	Norac (Canada)
Yakima Chief (United States)	Aroma Tech OY (Finland)
Carlton United Breweries (United Kingdom)	Quest (Holland)
English Hops (United Kingdom)	Wells Investment Ltd. (New Zealand)
Steiner Hops (Germany–United Kingdom–USA)	Lavipharm (Greece/USA)
US Nutraceuticals (United States)	GreenTek 21 (South Korea)
Supetrae (Denmark)	Eiffel (Australia)
Ferro Corporation (United States)	Arkopharma (France)

There also exists a cadre of companies that specialize in research and development and take refining up to a certain scale. Among the more prominent ones are Phasex, Praxair, Norac, Marc Sims Inc., Separex–Hitex, Flavex, Critical Processes Ltd., Bradford Particle Design (BPD), CPM Inc., Wells Investments Ltd., and Aphios Corp. These companies and a host of other organizations, consultancies, academia, and government laboratories can be useful sources for technical consultation/information.

What is a typical extraction/processing plant like in terms of scale? This is hard to quantify without picking what might be a "typical" example, but it is worth citing the NATECO$_2$ production facilities in Wolnzach, Germany as an example of a technically sophisticated and diverse operation. Some of its stated capacity and capabilities are as follows:

Production Plant #1: 4 × 2-m^3 extraction vessels
Laboratory Plant #2: 1000-mL countercurrent column
Production Plant #3: 4 × 4-m^3 extraction vessels
Production Plant #4: 3 × 500-L vessels
Pilot Plant #5: 2–200-L countercurrent column

This plant has historically focussed on the processing of hops but has diversified over the years to include the processing of other natural products, thereby providing for maximum utilization of the plant facilities.

To enumerate and discuss all of the nutraceutical or potential nutraceutical products that could be processed by critical fluids is beyond the scope of this review, particularly when one considers that in the past, many of these naturally derived products have been extracted with critical fluids. However, it is worth noting how these extracts are usually obtained in the SFE mode and what generic classes of materials have been extracted. For example, it is rare to obtain an extract without the occurrence of lipid coextractives; hence, the nutraceutical agent will frequently be diluted in a oily matrix. Examples of this type of extract are the previously mentioned tocopherol-containing extracts as well as natural pigments, such as the carotenoids (53). Tocotrienols can also be isolated via SFE as a complex from barley brand; likewise, the newer sterol or steryl ester concentrates can be obtained as mixtures from corn oil, rice brand oil, or saw palmetto sources. Many of these extracts containing other helpful nutraceutical ingredients, such as fatty acids, squalene, and so forth, can act in a synergistic mode with the principle nutraceutical agent.

It is worth noting that many current oil extraction and refining methods remove valuable nutraceutical components from the oil in the name of appearance and flavor. By-products and streams from these milling and refinement steps often contain preconcentrated sources of nutraceuticals (e.g., deodorizer distillate, residual protein meals, fibrous materials). Sources such as corn gluten meal, corn brand fiber, and alfalfa leaf protein concentrate have been extracted with SC-CO_2 successfully for their pigment, sterols, and fatty acid content. The high tocopherol–sterol–squalene content of deodorizer distillate is well known and several schemes employing critical fluids have been reported for fractionating this material to achieve higher–purity materials (54). Another processing agent that is a concentrator for nutraceutical components are the bleaching sorbents used by the vegetable oil processing industry. King and co-workers (55) have shown that very high oil yields can be obtained from clay bleaching earths; however, there is a need for the resultant extract to be analyzed for the enriched content of the nutraceutical components.

Critical fluid extraction has been applied for sometime now in the extraction of speciality oils, such as evening primrose, borage, black currant, and flax. These moieties contain the presence of γ-linoleic acid, a component which has been implicated favorably for the treatment of several medical conditions. Other specialized oils that have also been extracted with SC-CO_2 are wheat germ, avocado, sea buckthorn, sorghum brand/germ oat, and amaranth. Recently, there has been some studies employing critical fluids to obtain oil from fungi or marine sources, like spirulina, which are devoid of cholesterol. Partial deoiling has also been performed using SC-CO_2, more with respect to developing a functional food ingredient that has less fat (oil) or cholesterol content (i.e., low-calorie peanuts). Such deoiling can be done using either SC-CO_2 or propane and still meet the criterion for use in functional food products.

As has been stated previously, PPLs and many of the traditional herbal-medicine-type components are amenable to critical fluid extraction provided that GRAS cosolvents are employed along with SC-CO_2. Pure phosphatidylcholine and phosphatidylserine are finding widespread use, the latter in improving cognitive function; hence, the challenge for those using critical fluid processing for PPL recovery is to develop purification techniques for these naturally derived chemicals. Other nutraceutical agents that can be obtained similarly include extracts from chamomile, paprika, feverfew and gingko biloba (analytical studies), garlic, and ginger. Most of the common spices and mint oils, including a commercially available extract of rosemary, can be obtained via SC-CO_2 extraction.

For the above-mentioned products, critical fluid technology faces stiff competition from molecular (vacuum) distillation techniques, a time-honored technique, although greater selectivity is potentially available utilizing critical-fluid-based methods. This, coupled with the fact that CO_2-derived extracts exhibit, in many cases, extended shelf-lives due to the prophylactic action of the residual, nonoxidative CO_2 atmosphere, as well as micro-organism destruction due to exposure at higher pressures and temperatures, argues for a bright future for critical fluid technology in the nutraceutical marketplace.

6. IMPROVEMENT OF NUTRACEUTICAL FUNCTIONALITY THROUGH PROCESSING WITH CRITICAL FLUIDS

It is obvious from the discussion in the previous sections that many nutraceutical components or compositions are soluble to varying degrees in critical fluid media, particularly SC-CO_2. However, unlike pharmaceutical compounds, which are usually highly purified solutes dissolved in SC-CO_2 and/or cosolvent mixtures, nutraceutical components and compositions tend to be ill-defined and molecularly complex mixtures, where components often interact synergistically to induce a therapeutic benefit. Testimony to this complexity is provided by the capillary SFC profile of sawtooth palmetto berry extract provided in Figure 12, where the predominate fatty acid mixture along with low levels of sterols help alleviate prostate conditions in men. Obviously, sawtooth palmetto berry extract is quite different than a high-purity pharmaceutical, where perhaps only one or two (i.e., chiral racemates) major components are quantified by chromatography.

As noted at the start of the chapter, the strategy in the nutraceutical or functional food market is to fortify specific foods or natural products with ingredients that have, or are perceived to have, health-promoting benefits. Many of these products are orally ingested and, like pharmacological drugs, can be made effective more rapidly by rapid dissolution within the body. Therefore, it behooves us to consider the benefits of producing nutraceuticals by the processes employed to make small-diameter particles of medicinally active compounds for the pharmaceutical industry using the critical-fluid-based processes described earlier in this book. This can incorporate techniques like GAS (gas anti-solvent), RESS (rapid expansion of a supercritical solution), PCA (precipitation with compressed fluid anti-solvent), and so forth to produce small particles having low polydispersity, or encapsulation of nutraceutical ingredients and impregnation of an active ingredient into a food matrix (i.e., to make a functional food).

Although this would appear to be a good strategy, it is important to consider economics as they relate to the difference between the pharmaceutical and food industries. The food industry tends to be a commodity-driven marketplace with a low return margin on its products. The high hourly production rates characteristic of the food industry are a challenge given the current state of the art of particle formation technology employing supercritical fluids. However, consumers of nutraceuticals and functional foods tend to be willing to purchase these (nutraceutical) more expensive products, so it is not unreasonable to consider the benefit of applying critical-fluid-based techniques to produce ingredients via the methods noted in the previous paragraph. For example, consider the case for winning lecithin, or a more highly purified form of phospholipid concentrate from soybeans. Isolating lecithin from soybean oil or meal can yield a nutraceutical-grade lecithin that sells for $31 a pound. This is a significant mark up versus the price of soybean oil, which might be $0.25 a pound. Hence, when one considers a nutraceutical-grade phospholipid concentrate (concentrated phosphatidycholine or phosphatidyserine), the price is $1500 a pound. Obviously, with these economic incentives, the possibility of applying critical-fluid-based particle production processes may be justified.

The fundamental principles and methodology of producing fine particles, encapsulates, and so forth using critical fluid processing have been covered in depth in previous chapters, and it is not our intention to cover this as we conclude this chapter. However, it is worth considering a few of the possibilities in a generic sense and examining one application of the technology to a specific nutraceutical component or product. Table 9 lists applications of critical fluid technology reported at the 6th International Symposium on Supercritical Fluids in 2003, which have implications for the nutraceutical and/or functional food industry. As one can see, a number of the processes described in previous chapters have been applied to foodstuffs, nutraceutical ingredients or a suitable surrogate (e.g., cholesterol for sterols or sterol esters), or delivery systems that utilize liposomes or biodegradable natural polymers. By controlling the particle size, morphology, or method of delivery (encapsulation), these critical-fluid-based processes can produce a pleathora of materials, as noted in the application column. This includes specific chemicals utilized in nutraceuticals, such as high-antioxidant-containing spices, sterol esters, carotenoids, phospholipids, and fatty acids. This is ample evidence that such processes could impact on the functional food industry, as well as provide new food materials for the integration into the conventional food product chain.

We noted previously the high value and application of phospholipids-containing materials, like lecithin or phospholipid-base liposomes, as functional food ingredients. This is worth examining in greater detail, starting with the historical research of Quirin, Eggers, and Wagner (16,57,58) involving the jet extraction of lecithin from soybean oil. These researchers developed a continuous method for deoiling lecithin obtained from caustic or physical refining–derived lecithin feedstocks that resulted in powdered extracts, somewhat analogous to the lecithin- or phospholipid-based powders noted in Table 9. This process overcame some of the physical difficulties in refining lecithin by SFE with SC-CO_2 or SC-CO_2–csolvent mixtures due to the gelatinous nature of the lecithin feedstock. Further, the method referred to as "jet extraction" provided a more environmentally benign process than using propane for deoiling lecithin feedstocks.

TABLE 9 Materials Processing Using Critical Fluid Media with Implication or Direct Application in the Nutraceutical or Functional Food Industry

Process	Application	Ref.[a]
Concentrated powder form (CPF), jet dispersion	Dispersion of paprika soup powders	Weidner, pp. 1483–1495
Semicontinuous gas antisolvent process	Cholesterol morphology and precipitation	Subra and Vega, pp. 1629–1634
Rapid expansion of supercritical solution	Phystosterol micronization	Jiang et al., pp. 1653–1658
DELOS crystallization	Crystallization and control of stearic acid morphology	Ventosa et al., pp. 1673–1676
CPF Process	Controlled release of flavors and vitamins	F. Otto et al., pp. 1707–1712
Rapid expansion of supercritical solution	Encapsulation of β-sitosterol in low molecular weight polymer matrix	Turk et al., pp. 1747–1752
Supercritical antisolvent process	Incorporation of cholesterol or proteins in biodegradable matrix	Pellikaan, pp. 1765–1770
PGA and GAS processes	β-Carotene precipitation	Miguel et al., pp. 1783–1788
Particle from gas saturated solution (PGSS)	Lipid micronization of phosphatidylcholine and Tristearin	Elvassore et al., pp. 1853–1858
Supercritical antisolvent process	Biodegradable polymers precipitation studies with	Vega-Gonzalez, pp. 1877–1882

[a] The listed authors and inclusive page numbers all appear in Ref. 56.

The basis of jet extraction is that it facilitates the dispersion of a thin filet of lecithin into a highly turbulent jet of SC-CO_2. This is made possible by the use of two overlapping capillary jets. The lecithin is fed into the inside capillary, and the CO_2 enters into the larger outside capillary encasing the smaller-diameter capillary tube. The compressed CO_2 then mixes with the lecithin extrudate in a mixing chamber under conditions of high turbulence, affecting the deoiling of the lecithin and formation of a powdered product. The physicochemical basis of this separation process in terms of its effect on lecithin viscosity and interfacial tension have been reported by Eggers and Wagner (57,58) and resulted in the conceptualization of a continuous processing plant based on the above principle. Other processing options for lecithin with critical fluids are nicely summarized in Stahl et al.'s book (16).

A device similar in principle to the German researchers has been described by King (34) as shown in Figure 13. Figure 13a shows the general and initial design of the jet extraction system, and Figure 13b provides more critical details. In this laboratory-scale apparatus, the solids collection reservoir and two collector vessels were each 30.5 × 2.54-cm 316 stainless-steel tubing. The lecithin sample to be extracted is placed into the solids reservoir and extruded into the jet tube assembly with the aid of a nitrogen pressure head. This "pusher" gas flow rate is regulated by a micrometering valve.

As described earlier, the SC-CO_2 interfaces with the lecithin sample in the jet tube assembly (Fig. 13b): It is critical that in the pictured three-way valve that the viscous sample be injected through the 0.16-cm capillary into the larger concentric tube to avoid viscous back-streaming and to assure intimate contact with the SC-CO_2. The solubilized oil components are then routed through the back-pressure relief valve, where the CO_2 decompression occurs, resulting in the precipitation of the oily constituents in the liquid collector. The deoiled lecithin powder then drops into the solids collection vessel. Careful control must be exercised over the relative flow rates of the pushing and extraction fluids so as to maximize the contact time between the lecithin and the extraction fluid. This can also be amplified by using longer extraction chambers, which provide a long contact, or drop time, of the lecithin in the compressed CO_2 atmosphere.

Other notable advancements have been reported in the literature which use critical fluids for producing micron-size phospholipids-based powders as well as the formation of PPL-base liposomes. For example, Castor at Athios Corporation has patented several concepts (the CFL Process) (59,60), which permit the encapsulation of hydrophobic drugs as well as naturally derived drugs, such as taxol. The basis of these patents are that PPLs deposit out at the phase boundary as the PPL–drug aqueous phase (or multilamellar vesicle) undergoes decompression in a critical fluid atmosphere. The resulting liposomes formed in the presence of SC-CO_2 and other alternative fluids showed stability lifetimes exceeding 6 months, and this could be amplified by the inclusion of α-tocopherol in the PLL matrix, which provides prophylactic protection in an extending the lifetime of the resultant liposome.

Similar studies have also been performed by the research group of Charbit in Marseilles (61,62) in which fine PPL particles were formed by decompression using the SAS process. The focus of this research was to develop drug delivery systems, but it would equally applicable to the functional food area. Typically, a 2 wt% solution of soy-derived lecithin is

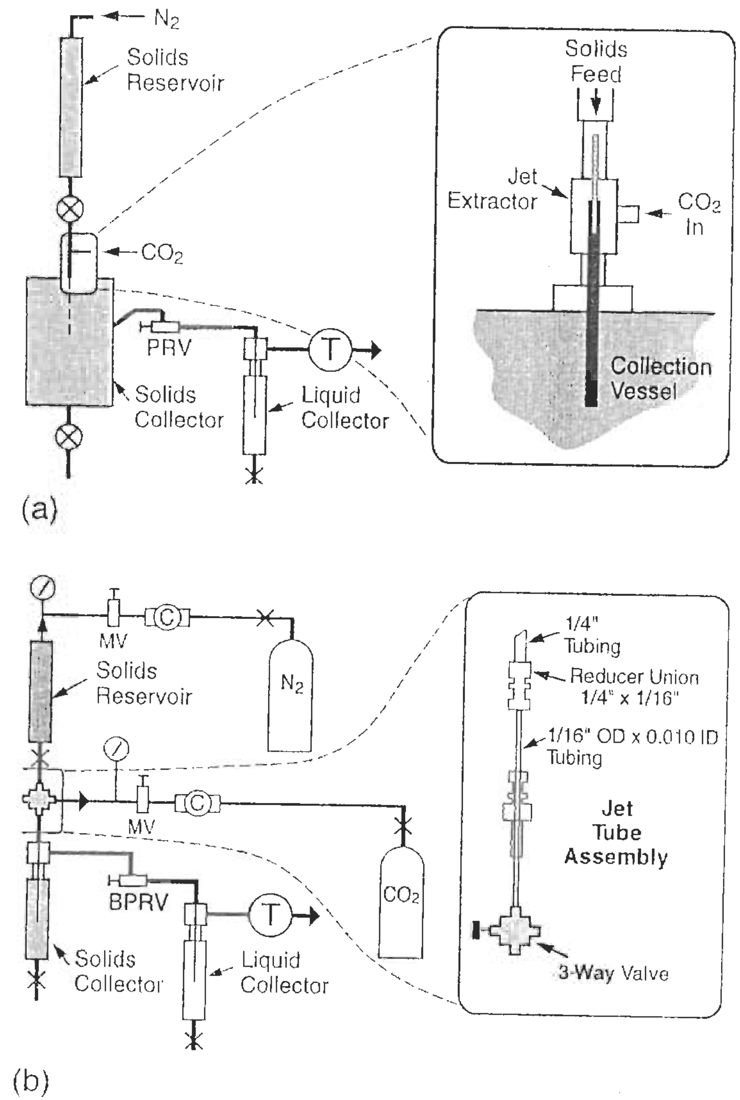

FIGURE 13 Basic schematic of laboratory-scale jet extractor system. PRV = pressure regulating valve; T = flow totalizer. (b) Details of jet extractor for deoiling soya lecithin. BPRV = back-pressure regulating valve; T-flow totalizer.

dissolved in an ethanol solution which is subsequently injected into SC-CO_2. Typical precipitation temperatures and pressures were 35°C and 8–11 MPa, respectively. The micronized PPL particles tended to be in the range 15–60μ, were amorphous in nature, and coalescenced when they were exposed to air.

Other schemes have been cited in the literature which employ phospholipids-based materials and supercritical fluids for fine-particle formation or encapsulation. Weber et al. (63) recovered lecithin from egg yolk extracts and induced crystallization by implementing the GAS process. Likewise, Frederiksen et al. (64) developed a new method of preparing liposomes to encapsulate water-soluble substances with the aid of ethanol. In this study, liposomes could be formed from phosphatidylcholine having 40–50-nm dimensions. A mixing process (ESMIC Process) (65) involving supercritical fluids has also reported, which is conducted in a stirred autoclave, to provide embedded pharmaceutical preparations in a lecithin matrix. Final particle size is partially control by the milling process taking place in the stirred autoclave containing the supercritical fluid medium.

In summary, the above studies and processes show the versatility in utilizing critical fluid media for modifying a target nutraceutical for functional food use. In the case of PPLs, the above studies illustrate how they could be prepared for rapid dissolution in food formulations or to encapsulate them for sustained delivery rates, or, alternatively, to use PPL-based liposomes and so on to encapsulate nutraceutical ingredients. Obvious extensions to other target nutraceuticals follow, and application of the technology outside of its traditional niche in the pharmaceutical industry seemed assured.

7. SUMMARY

In summary, we have attempted in this review to provide some understanding of the basic concepts involved in the use of critical fluids and to explain how these fluids are now exploited for the production of nutraceutical and other naturally derived products. Several illustrative examples have been provided of processing concepts and equipment, from the laboratory scale through production plants. There now exists an extensive 30-year history of critical fluid processing technology upon which to draw, replete with many examples of components having nutraceutical value that have been already extracted, fractionated, and reacted in these dense fluids.

In the future, fine-particle production or food material modifications via critical fluid technology will join the other generic processing methods that involve the use of these novel fluids. As noted by King (66), an "all-green" processing platform is emerging involving the use of multiple environmentally benign fluids and combined unit processing applications. Such an integrated critical fluid processing platform will make maximum use of production-scale equipment, which undoubtedly will include facilities for particle production, tailored toward the nutraceutical marketplace.

REFERENCES

1. Koseoglu SS. Overview of nutraceuticals/functional foods. Nutraceuticals and Functional Foods Short Course, College Station, TX, 2000.
2. King JW, List GR, eds. Supercritical Fluid Technology in Oil and Lipid Chemistry. Champaign, IL: AOCS Press, 1996.
3. Mukhopadhyay M. Natural Extracts Using Supercritical Carbon Dioxide. Boca Raton, FL: CRC Press, 2000.
4. King JW. Supercritical fluid processing of cosmetic raw materials. Cosmet Toil 1991; 61:61–67.
5. Revercon E. Fractional separation of of SCF extracts from marjorum leaves: mass transfer and optimization. J Supercrit Fluids 1992; 5:256–261.
6. Krukonis VJ, Vivian JE, Bambara CJ, Nillson WB, Martin RA. Concentration of eicosapentaenoic acid by supercritical fluid extraction: a design study of a continuous production process. In: Flick GJ, Martin RE, eds. Advances in Seafood Biochemistry. Basel: Technomic Publishing Company, 1992:169–179.
7. Reverchon E, Marciano A, Poletto M. Fractionation of peel oil key mixture by supercritical CO_2 in a continuous tower. Ind Eng Chem Res 1997; 36:4940–4948.
8. King JW. Sub- and supercritical fluid processing of agrimaterials: extraction, fractionation, and reaction modes. In: Kiran E, ed. Supercritical Fluids—Fundamentals and Applications. Dordrecht: Kluwer, 2000:451–488.
9. Mohamed RS, Saldana MDA, Socantatype FH, Kieckbusch TG. Reduction in the cholesterol content of butter oil using supercritical ethane extraction and adsorption on alumina. J Supercrit Fluids 2000; 16:225–233.
10. Giese T, Johanssen M, Brunner G. Separation of stereoisomers in a SMB–SFC plant: determination of isotherms and simulation. In: Dahmen N, Dinjus E, eds. Proceedings of the International Meeting of the GVC-Fachausschuss Hochdruckverfahrenstechnik, 1999:283–286.
11. Nicolosi RJ, Ausman LM, Rogers EJ. New Technologies for Healthy Foods & Nutraceuticals. Shrewsbury, MA: ATKL Press, Inc., 1997:84.
12. Fitzpatrick KC. Sterol ester products face US hurdles. INFORM 1999; 10:172–176.
13. Anastas PT, Williamson TC, eds. Green Chemistry. Washington, DC: American Chemical Society, 1996.
14. Froning GW, Wehling RL, Cuppett SL, Pierce MM, Niemann L, Siekman DK. Extraction of cholesterol and other lipids from dried egg yolk using supercritical carbon dioxide. J Food Sci 1990; 55:95–98.
15. Passey CA. Commercial feasibility of a supercritical extraction plant for making reduced-calorie peanuts. In: Rizvi SSH, ed. Supercritical Fluid Processing of Food and Biomaterials. London: Blackie Academic, 1994:223–243.
16. Stahl E, Quirin K-W, Gerard D. Dense Gases for Extraction and Refining. Heidelberg: Springer-Verlag, 1987.

17. Anonymous. CO_2 extracts—food and medicine. Food Market Technol 1997; 11(4):27–31.
18. King JW, Johnson JL, Orton WL, McKeith FK, O'Connor PL, Novakofski J, Carr TR. Effect of supercritical carbon dioxide extraction on the fat and cholesterol content of beef patties. J Food Sci 1993; 58:950–952, 958.
19. Clifford AA, Basile A, Jimenez-Carmona MM, Al-Said SHR. Extraction of natural products with superheated water. Proceedings of the GVC-Fachausssschuss Hochdruckverfahrenstechnik, 1999:181–184.
20. Mannia M, Kim H, Wai CM. Supercritical carbon dioxide and high pressure water extraction of bioactive compounds in St. John's wort. Proceedings of the Super Green 2002 Symposium, 2002:74–78.
21. King JW, Grabiel RD, Wightman JD. Subcritical water extraction of anthocyanins from fruit berry substrates. In: Brunner G, ed. Proceedings of the 6th International Symposium on Supercritical Fluids—Vol. 1, 2003:409–418.
22. King MB, Bott TR, eds. Extraction of Natural Products Using Near-Critical Solvents. London: Blackie Academic, 1993.
23. Brogle H. CO_2 as a solvent: its properties and uses. Chem Ind 1982; 19:385–390.
24. Clifford AA, Basile A, Jiminez-Carmona MM, Al-Saidi SHR. Extraction of natural products with superheated water. Proceedings of the 6th Meeting on Supercritical Fluid Chemistry and Materials, 1999:485–490.
25. Basile A, Jimenez-Carmona MM, Clifford AA. Extraction of rosemary by superheated water. J Agric Food Chem 1998; 46:5205–5209.
26. Hawthorne SB, Yang Y, Miller DJ. Extraction of organic pollutants from environmental solids with sub- and supercritical water. Anal Chem 1994; 66:2912–2920.
27. King JW. Critical fluid options for isolating and processing agricultural and natural products. Proceedings of the Super Green 2002 Symposium, 2002: 74–78.
28. King JW, Eller FJ, Taylor SL, Neese AL. Utilization of analytical critical fluid instrumentation in non-analytical applications. Proceedings of the 5th International Symposium on Supercritical Fluids (CD disk).
29. Pietsch A, Eggers R. The mixer–settler principle as a separation unit in supercritical fluid processes. J Supercrit Fluids 1999; 14:163–171.
30. Nilsson WB. Supercritical fluid extraction and fractionation of fish oils. In: King JW, List GR, eds. Supercritical Fluid Technology in Oil and Lipid. Champaign, IL: AOCS Press, 1996:180–212.
31. King JW, Favati F, Taylor SL. Production of tocopherol concentrates by supercritical fluid extraction and chromatography. Sep Sci Technol 1996; 31:1843–1857.
32. Peper S, Cammerer S, Johannsen M, Brunner G. Supercritical fluid chromatography process optimization of the separation of tocopherol homolgues. In: Kikia I, Perrut M, eds. Proceedings of the 6th International Symposium on Supercritical Fluids, 1999:563–568.
33. Eggers R. High pressure extraction of oilseeds. J Am Oil Chem Soc 1985; 62:1222–1230.
34. King JW. Analytical-process supercritical fluid extraction: a synergistic

combination for solving analytical and laboratory scale problems. Trends Anal Chem 1995; 14:474–481.
35. King JW. Critical fluids for oil extraction. In: Wan PJ, Wakelyn PJ, eds. Technology and Solvents for Extracting Oilseeds and Non-petroleum Oils. Champaign, IL: AOCS Press, 1997:283–310.
36. List GR, King JW, Johnson JH, Warner K, Mounts TL. Supercritical CO_2 degumming and physical refining of soybean oil. J Am Oil Chem Soc 1993; 70:473–477.
37. Taylor SL, King JW, Montanari L, Fantozzi P, Blanco MA. Enrichment and fractionation of phospholipid concentrates by supercritical fluid extraction and chromatography. Ital J Food Sci 1999; 12:65–76.
38. Schultz WG, Randall JM. Liquid carbon dioxide for selective aroma extraction. Food Technol 1970; 24(11):94–98.
39. Robinson JR, Sims M. Method and system for extracting a solute from a fluid using dense gas and a porous membrane. US Patent 5,490,884 (1996).
40. Lee ML, Markides KE, eds. Analytical Supercritical Fluid Chromatography and Extraction. Provo, UT: Chromatography Conferences, 1990.
41. Taylor LT. Supercritical Fluid Extraction. New York: Wiley, 1998.
42. Luque de Castro MD, Valcarel M, Tena MT. Analytical Supercritical Fluid. Heidelberg: Springer-Verlag, 1994.
43. Dean JR, ed. Applications of Supercritical Fluids in Industrial Analysis. London: Blackie Academic, 1993.
44. King JW. Applications of capillary supercritical fluid chromatography–supercritical fluid extraction to natural products. J Chromatogr Sci 1990; 28:9–14.
45. King JW. Capillary supercritical fluid chromatography of cosmetic ingredients and formulations. J Microcol Sep 1998; 10:33–39.
46. Chandra A, Nair MG. Supercritical carbon dioxide extraction of daidzein and genistein from soybean products. Phytochem Anal 1996; 7:259–262.
47. Montanari L, Fantozzi P, Snyder JM, King JW. Selective extraction of phospholipids from soybeans with supercritical carbon dioxide and ethanol. J Supercrit Fluids 1999; 14:87–93.
48. Taylor SL, King JW. Supercritical luid fractionation of phospholipids. Abstracts of the 8th Int. Symposium on Supercritical Fluid Chromatography and Extraction, 1998.
49. Taylor SL, King JW, Snyder JM. Tandem supercritical fluid extraction/chromatographic studies of the desert botanical species, *Dalea spinosa*. J Microcol Sep 1994; 6:467–473.
50. King JW, Snyder JM. Supercritical fluid chromatography: a shortcut in lipid analysis. In: McDonald RE, Mossoba M, eds. New Techniques and Applications in Lipid Analysis. Champaign, Il: AOCS Press, 1997:139–162.
51. Snyder JM, Taylor SL, King JW. Analysis of tocopherols by capillary supercritical fluid chromatography and mass spectrometry. J Am Oil Chem Soc 1993; 70:49–354.
52. DeSwaef SI, Kleibohmer W, Vlietinck AJ. Supercritical fluid chromatography of free fatty acids and ethyl esters in ethanolic extracts of sabal serrulata. Phytochem Anal 1996; 7:223–227.

53. Favati F, King JW, Friedrich JP, Eskins K. Supercritical extraction of carotene and lutein from leaf protein concentrates. J Food Sci 1988; 53:1532–1536.
54. Ssuss D, Brunner G. Countercurrent extraction with supercritical carbon dioxide: behaviour of a complex natural mixture. Proceedings of the GVC-Fachaussschuss Hochdruckverfahrenstechnik-, 1999:189–192.
55. King JW, List GR, Johnson JH. Supercritical carbon dioxide extraction of spent bleaching clays. J Supercrit Fluids 1992; 5:38–41.
56. Brunner G, Kikia I, Perrut M, eds. Proceedings of the 6th International Symposium on Supercritical Fluids, Tome 3, 2003.
57. Eggers R, Wagner H. Extraction device for high viscous media in a high-turbulent two-phase flow with SC-CO_2. J Supercrit Fluids 1993; 6:31–37.
58. Wagner H, Eggers R. Entolung von sojalecithin mit uberkritischen kohlendioxid in hochturbulenter zweiphasestromung. Fat Sci Technol 1993; 95:75–80.
59. Castor TP, Chen L. Method and apparatus for making liposomes containing hydrophobic drugs. US patent 5,776,486, July 7, 1998.
60. Castor TP. Methods and apparatus for making liposomes. US patent 5,554,382, September 10, 1996.
61. Magnan C, Commenges H, Badens E, Charbit G. Fine phospholipid particles formed by precipitation with a compressed fluid anti-solvent. Proceedings of the GVC-Fachaussschuss Hochdruckverfahrenstechnik, 1999:223.
62. Magnan C, Badens E, Commenges N, Charbit G. Soy lecithin micronization by precipitation with a compressed fluid anti-solvent—influence of process parameters. J Supercrit Fluids 2000; 19:69–77.
63. Weber A, Nolte C, Bork M, Kummel R. Recovery of lecithin from egg yolk-extracts by gas anti-solvent crystallization. Proceedings of the 6th Meeting on Supercritical Fluids: Chemistry and Materials. 181–184.
64. Frederiksen L, Anton K, Barrat BJ, VanHoogevest P, Lenenberger H. Proceedings of the 3rd International Symposium on Supercritical Fluids, Tome 3, 1994:235–240.
65. Heidlas J, Zhang Z. New approaches to formulate compounds using supercritical gases. Proceedings of the 7th Meeting on Supercritical Fluids, Tome 1, 2000:167–172.
66. King JW. Critical fluid options for isolating and processing agricultural and natural products. Proceedings of SuperGreen, 2002:61–66.

15

Scale-Up Issues for Supercritical Fluid Processing in Compliance with GMP

Jean-Yves Clavier and Michel Perrut
Lavipharm, East Windsor, New Jersey, U.S.A. and Separex 5, Champigneulles, France

Although very important research and development (R&D) means are dedicated to applications of supercritical fluids (SCFs) in the pharmaceutical industry, a very limited number of commercial plants are now operating or under construction and few companies have acquired some know-how in process scale-up, especially in SCF formulation and particle design, in compliance with the constraints imposed in this industry [traceability and Good Manufacturing Processes (GMP), sterility, etc].

In this chapter, based on our experience gathered when building tens of SCF plants during the past decade, including some for processing clinical lots in compliance with GMP, we will try to present both the general scale-up rules applicable to large-scale SCF plant construction, operation, and maintenance, the specific scale-up know-how related to pharmaceutical plants, and especially those dedicated to drug formulation and particle design.